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Dephosphonylation of protected deoxynucleoside and oligodeoxynucleotide *H*-phosphonates

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Abstract

The conversion of four 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyribonucleoside 3′-*H*-phosphonates **1** (B=**4**, **5**, **6** and **7**) into their partially-protected nucleoside precursors **3** (B=**4**, **5**, **6** and **7**, respectively) in good isolated yields is described. The procedure used is also suitable for the dephosphonylation of protected oligonucleotide *H*-phosphonate blocks. © 1999 Elsevier Science Ltd. All rights reserved.

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For a number of years, appropriately protected 2′-deoxyribonucleoside 3′-*H*-phosphonates **1** have been used successfully as building blocks in the solid phase synthesis of oligonucleotides.[1] More recently, we have developed[2] a solution phase synthesis of oligonucleotides and their phosphorothioate analogues that is also based on *H*-phosphonate building blocks. Although the excess of the *H*-phosphonate component required[2] in solution phase synthesis is generally only ca. 20%, recovery of the excess material would become a matter of considerable importance if the process were to be scaled-up appreciably. Clearly, recovery becomes an even more important matter when oligonucleotide *H*-phosphonates (see below) rather than monomeric building blocks are involved. In order to facilitate the recovery and purification of the excess *H*-phosphonates (e.g. **1**), we have developed a procedure (Scheme 1) for their dephosphonylation to give the corresponding hydroxy compounds (e.g. **3**), which are uncharged and therefore much easier to isolate in a pure state.

We now report that when 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyribonucleoside 3′-*H*-phosphonates[3] **1** are treated (Scheme 1) with an excess each of ethylene glycol and pivaloyl chloride in pyridine solution, they are quantitatively converted into the corresponding 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxynucleoside derivatives **3**. For example, when triethylammonium 5′-*O*-(4,4′-dimethoxytrityl)thymidine-3′-*H*-phosphonate **1**; B=**7** was allowed to react with a fourfold excess of ethylene glycol and a threefold excess of pivaloyl chloride in a dry pyridine solution (Table 1, entry 4) at 0°C, it was quantitatively converted into 5′-*O*-(4,4′-dimethoxytrityl)thymidine **3**; B=**7** within 20 min. Following work-up of the

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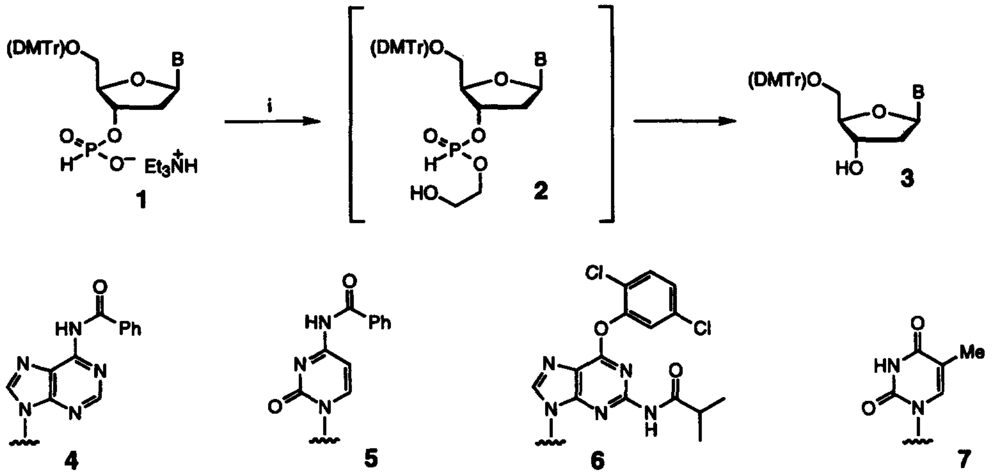
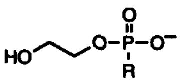
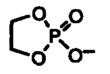


Scheme 1. Reagents and conditions: (i) $HOCH_2CH_2OH$, Me_3CCOCl, C_5H_5N, 0°C, 20 min

products,[4] the pure nucleoside derivative **3**; B=**7**, which was identical to authentic material, was isolated in 91% yield. In the same way, the other three *H*-phosphonate building blocks **1** (B=**4**, **5** and **6**) used in our solution phase synthesis of oligodeoxyribonucleotides and their phosphorothioate analogues[2] were quantitatively converted into their partially-protected deoxynucleoside precursors **3** (B=**4**, **5** and **6**, respectively), which were then isolated in good yields (Table 1, entries 1–3). Finally, the trimer 3′-*H*-phosphonates, DMTr-*Gp*(*s*)*Cp*(*s*)Tp(H) and DMTr-*Gp*(*s*)*Cp*(*s*)*G*p(H) (Table 1, entries 5 and 6; see footnote **b** for an explanation of the abbreviations), two of the protected trimer 3′-*H*-phosphonates that have been used in a block solution phase synthesis of an 'antisense' 21-mer oligodeoxyribonucleotide phosphorothioate,[5] were converted into their protected trinucleoside di-phosphorothioate precursors (DMTr-*Gp*(*s*)*Cp*(*s*)*T*-OH and DMTr-*Gp*(*s*)*Cp*(*s*)*G*-OH) in 89 and 90% isolated yields, respectively.



8 a ; R = OR
b ; R = H



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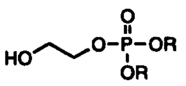

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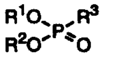
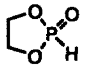


11 a ; R³ = H
b ; R³ = OR



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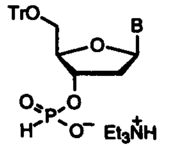

13 ; B = **7**

Not surprisingly, the putative intermediate *H*-phosphonate diesters **2** were not detected in the products of any of the above reactions. Alkyl 2-hydroxyethyl phosphates **8a** readily undergo alkaline hydrolysis[7] via ethylene cyclic phosphate **9** and corresponding uncharged dialkyl 2-hydroxyethyl phosphates[8] **10** presumably undergo base-catalyzed cyclization much more readily still. Furthermore, as dialkyl *H*-phosphonates **11a** are known[9] to undergo alkaline hydrolysis at a rate some 5 orders of magnitude faster than related trialkyl phosphates **11b**, *H*-phosphonate diesters such as **2** would be expected to fragment to give the corresponding nucleoside derivatives **3** and ethylene cyclic *H*-phosphonate **12** virtually instantaneously under the reaction conditions. Due to ring strain[7] and the inherent reactivity of *H*-phosphonate

Table 1

Dephosphonylation reactions[a]

Entry No.	Substrate	Scale (mmol)	Product	Isolated Yield (%)
1	**1**; B = **4**	2.0	**3**; B = **4**	87
2	**1**; B = **5**	2.0	**3**; B = **5**	91
3	**1**; B = **6**	2.0	**3**; B = **6**	91
4	**1**; B = **7**	2.0	**3**; B = **7**	91
5	DMTr-*Gp(s)Cp(s)T*p(H)[b]	0.165	DMTr-*Gp(s)Cp(s)T*-OH[b]	89
6	DMTr-*Gp(s)Cp(s)G*p(H)[b]	0.425	DMTr-*Gp(s)Cp(s)G*-OH[b]	90

[a] See References and Notes (ref. 4) for experimental details.

[b] *C* and *G* represent base-protected (as in **5** and **6**, respectively) 2'-deoxycytidine and 2'-deoxyguanosine residues; *T* represents a 4-*O*-phenyl-protected thymidine residue;[6] -*p(s)*- represents an *S*-(2-cyanoethyl)-protected phosphorothioate triester; -p(H) represents an *H*-phosphonate monoester function. See ref. 2 for a fuller explanation of these abbreviations.

diesters,[9] ethylene cyclic *H*-phosphonate **12** would be expected to undergo hydrolytic cleavage to give 2-hydroxyethyl *H*-phosphonate **8b** under extremely mild conditions. In order to obtain some evidence as to the fate of the *H*-phosphonate moiety, we followed the reaction between triethylammonium 5'-*O*-tritylthymidine 3'-*H*-phosphonate **13**; B=**7** (0.25 mmol), ethylene glycol (1.0 mmol) and pivaloyl chloride (0.75 mmol) in pyridine-d_5 (0.5 ml) at 0°C by ^{31}P NMR spectroscopy. After 3 min, no substrate **13**; B=**7** (δ_P [C_5D_5N] 2.7) remained and no change in the NMR spectrum of the products was observed after a further period of 12 min. The strongest signal (δ 11.4, $J_{P,H} \sim 712$; ca. 70%) in the spectrum could conceivably be assigned to the phosphorus resonance of the cyclic *H*-phosphonate **12**. Weaker resonance signals were observed at δ 5.5 ($J_{P,H} \sim 630$; ca. 10%) and δ 9.7 (ca. 9%).

After this study had been completed, other workers described[10] the conversion of two protected ribonucleoside 3'-*H*-phosphonates into the corresponding protected ribonucleoside derivatives by treatment with approximately stoichiometric quantities of glycerol and 1-adamantanecarbonyl chloride in pyridine solution. These workers also indicated[10] that the latter two reagents could be replaced by ethylene glycol and pivaloyl chloride, respectively.

Acknowledgements

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4. The following procedure was used for the reactions in Table 1, entries 1–4. Pivaloyl chloride (0.74 ml, 6.0 mmol) was added to a stirred, dry solution of the substrate (2.0 mmol) and ethylene glycol (0.45 ml, 8.0 mmol) at 0°C (ice-water bath). After 20 min, saturated aqueous sodium hydrogen carbonate (5 ml) was added and the products were partitioned between dichloromethane (30 ml) and saturated aqueous sodium hydrogen carbonate (20 ml). The layers were separated and the aqueous layer was back extracted with dichloromethane (2×20 ml). The combined organic extracts were washed

with saturated aqueous sodium hydrogen carbonate (2×20 ml), dried ($MgSO_4$) and evaporated under reduced pressure. A solution of the residue in dichloromethane (3 ml) was added dropwise to stirred petroleum ether (b.p. 30–40°C, 35 ml) to give the product (entries 2, 3 and 4) as a colourless precipitate that was collected by filtration. The other products (Table 1, entries 1, 5 and 6) were purified by short column chromatography on silica gel. The same stoichiometry (with respect to the excess of ethylene glycol and pivaloyl chloride) was used in reactions in entries 5 and 6. However, proportionally smaller volumes of solvents and aqueous sodium hydrogen carbonate were used.

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